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Eukaryotic translational control: Adeno-associated virus protein synthesis is affected by a mutation in the adenovirus DNA-binding protein

(parvovirus/RNA splicing/protein synthesis *in vivo* and *in vitro*/protein processing/translational control)

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ABSTRACT **Growth of adeno-associated virus (AAV) requires expression of certain adenovirus (Ad) genes in the same cell. AAV particles contain three proteins, VP1 (M_r 85,700), VP2 (M_r 72,000), and VP3 (M_r 61,500). These proteins have overlapping peptide maps. We recently reported that AAV RNAs make up a 3′-coterminal family of overlapping molecules. We report here that the most abundant AAV mRNA, a 2.3-kilobase spliced RNA, codes for all three proteins—VP1, VP2, and VP3—when translated in an *in vitro* reticulocyte lysate. This shows that the AAV capsid proteins are coded by the genome sequence between map positions 48.0 and 96.0 (1 map unit is 1% of the genome or 47 base pairs). When AAV was grown in human KB cells with the Ad temperature-sensitive mutant Ad5ts125 at the nonpermissive temperature (40°C), the accumulation *in vivo* of AAV capsid proteins VP1, VP2, and VP3 was decreased to less than 1/50th. However, normal amounts of the 2.3-kilobase mRNA were accumulated, and this RNA could be efficiently translated in an *in vitro* reticulocyte lysate system to yield VP1, VP2, and VP3. These experiments suggest that in infected cells control is exerted upon the AAV 2.3-kilobase mRNA at the translational level and that this control can be influenced by mutations in Ad. These Ad mutations map in the region 2 early gene for the Ad DNA-binding protein. The temperature-sensitive system that we have studied may be useful for analysis of translational control of a eukaryotic mRNA.**

Adeno-associated virus (AAV) is a defective parvovirus. Growth of AAV has an absolute requirement for expression of certain adenovirus (Ad) or herpesvirus genes in the same cell. AAV particles consist of a 4.7-kilobase (kb) linear single-stranded DNA genome packaged in a protein capsid. The protein capsid is composed of one major (about 85% by mass) M_r 61,500 protein, VP3, and two minor proteins (each about 7% by mass) called VP2 (M_r 72,000) and VP1 (M_r 85,000–90,000), respectively (1). These three AAV proteins, which exhibit immunological crossreactions (2, 3) and overlapping peptide maps (ref. 4; unpublished), are specified by AAV mRNA (5).

We recently reported that cytoplasmic poly(A)-containing AAV RNA species make up a family of overlapping spliced RNAs of 2.3, 3.3, 3.6, and 3.9 kb (6). In addition, the analogous coterminal unspliced AAV RNA transcripts were observed in both the nucleus and cytoplasm (7, 8). The 2.3-kb spliced RNA is by far the most abundant on a molar basis and accounts for at least 50% of all the cytoplasmic AAV transcripts (6). We have translated cytoplasmic AAV RNA in an *in vitro* reticulocyte lysate and report here that the 2.3-kb spliced RNA codes *in vitro* for all three AAV capsid proteins, VP1, VP2, and VP3.

The nature of the helper functions for AAV growth provided by either adenoviruses or herpesviruses are not clearly known. Current evidence strongly indicates that for adenovirus these helper functions are controlled by a subset of Ad early genes (9, 10). We have shown that in human KB cells the Ad temperature-sensitive mutant Ad5ts125 at the nonpermissive temperature (40°C) is an inefficient helper of AAV (9). This Ad mutant has a lesion located near the carboxyl terminus of the M_r 72,000 DNA-binding protein coded for by the early region 2 gene (11–13). One defect in the AAV helper ability of this Ad mutant is manifested in the failure to accumulate the larger spliced AAV RNAs, although a normal amount of the 2.3-kb spliced RNA is accumulated (9). In addition, AAV capsids fail to accumulate when the mutant helper is used at 40°C. We report here that the 2.3-kb AAV RNA isolated from cells coinfected with mutant or wild-type Ad have similar biological activity when tested in the *in vitro* translation system.

These experiments suggest that there is a control at the translational level exerted upon the AAV 2.3-kb mRNA and that this control can be influenced by mutations in Ad. Thus AAV, together with a mutant Ad, may provide a temperature-sensitive system that will be useful in analyzing translational control of a eukaryotic mRNA.

METHODS

Cells and Viruses. Stocks of AAV type 2 (AAV2), wild-type adenovirus 5 (Ad5wt), or the temperature-sensitive mutant Ad5ts125 (11) were grown and assayed as described (9). The nonpermissive temperature for the mutant was 40°C. For all the experiments reported here KB cells grown either in monolayer or spinner culture were coinfected with AAV and Ad as described (9).

Preparation and Fractionation of Cytoplasmic RNA. KB cells in spinner culture were infected with AAV2 and either Adwt or Ad5ts125 and grown at 40°C. At 20 hr after infection, cytoplasmic poly(A)-containing RNA was isolated as described (14). For size fractionation, a sample (about 100 μg) of RNA was dissolved in 200 μl of a buffer containing 37.5% formamide (vol/vol), 10 mM Tris·HCl at pH 7.5, 10 mM NaCl, 1 mM Na_2EDTA, and 0.1% $NaDodSO_4$. The sample was incubated at 40°C for 3 min, then cooled to room temperature and layered on a 17-ml gradient containg 15–30% sucrose (wt/vol), 100 mM NaCl, 10 mM Tris·HCl at pH 7.5, 1 mM EDTA, and 0.5% $NaDodSO_4$. Gradients were centrifuged in polyallomer tubes in the SW 27 rotor of a Beckman ultracentrifuge at 26,000 rpm for 2 hr at 20°C. Fractions (430 μl) were collected from the bottom of the tube. The positions of 28S, 18S, and 4–5S RNA in the gradients were determined by sedimentation of ^{3}H-labeled

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Abbreviations: AAV, adeno-associated virus; Ad, adenovirus; kb, kilobase(s); IU, infectious units; PFU, plaque-forming units.

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cytoplasmic RNA in a parallel sucrose gradient. RNA was recovered from the gradient fraction by precipitation at −20°C after addition of 10 μg of purified *Escherichia coli* tRNA, NaCl to a final concentration of 0.25 M, and ethanol (2.5 vol). The RNA recovered from each fraction was resuspended in sterile water (10 μl) and stored at −80°C.

Electrophoretic Analysis of AAV RNA. AAV RNA was analyzed by using the procedures of Berk and Sharp (15) as described (6). Briefly, RNA was annealed with denatured AAV [^{32}P]DNA in conditions that favor DNA·RNA hybridization. The duplex nucleic acid structures formed were then digested with endonuclease S1, electrophoresed on 1.4% agarose gels at neutral pH, and visualized, after the gel was dried, by fluorography using a Du Pont Cronex lightning plus intensifying screen at −80°C.

***In Vitro* Translation of RNA.** Cytoplasmic poly(A)-containing RNA obtained from sucrose gradient fractions was translated *in vitro* in a messenger-dependent rabbit reticulocyte system (16) using [^{35}S]methionine (600 Ci/mmol; 5 mCi/ml; 1 Ci = 3.7 × 10^{10} becquerels) as the radioactive label at a concentration of 2 μl per 12.5 μl of translation reaction mixture.

***In Vivo* Labeling of AAV Protein.** Monolayers of KB cells were incubated from 12 to 30 hr after infection in medium containing methionine at 1.5 μg/ml and supplemented with [^{35}S]methionine (900–1100 Ci/mmol) at 100 μCi/ml final concentration. Labeled proteins were then prepared by lysing the cells as described before (17).

Analysis of Virus Proteins. Anti-AAV2 IgG [40% saturated $(NH_4)_2SO_4$] fraction was prepared from rabbits that were hyperimmunized with purified AAV2 full particles. Proteins labeled with [^{35}S]methionine were incubated with anti-AAV2 IgG or normal rabbit IgG. The antigen–antibody complexes were then collected by precipitation with inactivated *Staphylococcus aureus* (Cowan I strain) cells (18). The precipitates were solubilized by boiling in $NaDodSO_4$/2-mercaptoethanol (17) and analyzed in a $NaDodSO_4$/polyacrylamide gel system (19) according to O'Farrell *et al.* (20). Radiolabeled protein bands on gels were detected by fluorography (21).

RESULTS

Identification of the AAV mRNA that Codes for Capsid Proteins. The structures of the steady-state RNA transcripts derived from AAV DNA and present in cells at 16–20 hr after infection are shown schematically in Fig. 1. The 2.3-kb spliced RNA is the most abundant AAV RNA species (more than 50% of the total). The coterminal 3.6-kb spliced and 3.9-kb unspliced RNAs with 5′ termini at map position 13 are the least abundant (usually less than 1% of total AAV RNA) and often are undetectable (unpublished). Although all the AAV RNA species shown in Fig. 1 were found in cytoplasmic poly(A)-containing RNA (6), the unspliced species are found predominantly in the nucleus and the spliced species are mainly in the cytoplasm (7, 8).

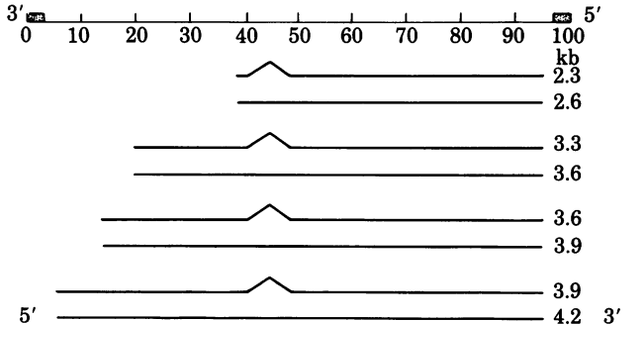

FIG. 1. Diagram of structure and map location of AAV mRNA species (6). The upper line represent the AAV DNA minus strand, calibrated in map units, and the stippled boxes show the approximate location of the terminal repetition. The lower lines represent the AAV mRNAs. The kinks indicate the intervening sequences removed by splicing. All the AAV mRNAs are complementary to the DNA minus strand.

Poly(A)-containing cytoplasmic RNA from cells infected with AAV and Ad5wt was fractionated by sedimentation in a neutral sucrose gradient after denaturation in formamide. RNA recovered from alternate gradient fractions was then analyzed by using the procedure of Berk and Sharp (15) to determine the distribution of AAV mRNAs. The 2.3-kb AAV RNA was clearly the predominant species and sedimented at about 20 S (Fig. 2A, fractions 13–15) whereas the unspliced 2.6-kb RNA sedimented slightly faster (Fig. 2A, fraction 11). The other larger AAV RNAs

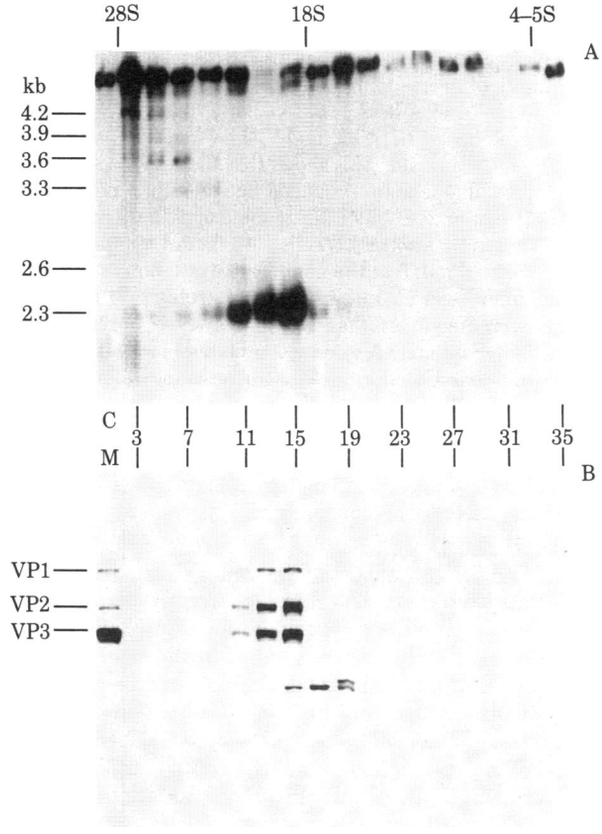

FIG. 2. Transcription and translation analysis of AAV RNA. Cytoplasmic poly(A)-containing RNA (100μg) from KB cells infected with AAV2 [10 infectious units (IU)/cell] and Ad5wt and grown at 37°C was isolated and sedimented in a neutral sucrose gradient. RNA from each fraction was recovered and dissolved in 10 μl of water. (*A*) Aliquots (4 μl) of RNA from odd-numbered fractions were incubated with heat-denatured AAV2 [^{32}P]DNA then digested with endodeoxyribonuclease S1 and electrophoresed at neutral pH in a 1.4% agarose gel (6, 15). The gel was visualized by autoradiography. The sizes of the digestion products were determined from restriction endonuclease fragments of AAV DNA electrophoresed in the same gel (not shown). Gradient fraction numbers are indicated between the upper and lower panels. Track C contained control yeast RNA. (*B*) Aliquots (2 μl) of RNA from odd-numbered fractions were translated *in vitro* in the reticulocyte lysate. ^{35}S-Labeled AAV proteins were detected by immunoprecipitation and electrophoresis in a 9% polyacrylamide gel. Track M contained ^{35}S-labeled capsid proteins from purified AAV particles. The tracks are numbered (3–35) according to the fraction numbers in the sucrose density gradient.

sedimented more rapidly and were partially fractionated according to size.

Samples of RNA from the same gradient fractions as those shown in Fig. 2A were also tested for their ability to direct *in vitro* synthesis of AAV proteins. As shown in Fig. 2B, RNA mainly in fractions 11–15 directed the synthesis *in vitro* of three ^{35}S-labeled polypeptides that were immunoprecipitated with anti-AAV2 capsid IgG and that co-electrophoresed with the three AAV capsid proteins, VP1, VP2, and VP3. In the gradient (Fig. 2) the distributions of the AAV mRNA activities for the synthesis of each of the three virus proteins were identical. Furthermore, the distribution was parallel to the distribution of the 2.3-kb RNA. We conclude that all three AAV capsid proteins are coded by the 2.3-kb RNA. The other AAV RNAs did not appear to code for any products that were immunoprecipitable by anti-AAV capsid IgG. There are two ^{35}S-labeled polypeptides of M_r 42,000–44,000 precipitated from reactions with RNA from fractions 17–19, but their distribution does not coincide with that of any discrete AAV RNAs. They may represent translation products from partially degraded AAV RNA or nonspecific precipitation of cellular proteins such as actin.

Effect of an Ad5 Early Region 2 Mutation on AAV Protein Synthesis. At the nonpermissive temperature of 40°C, the adenovirus mutant Ad5ts125 was an inefficient helper for AAV (9). This defect was assayed as a failure to accumulate AAV capsids. This is shown by the experiment in Fig. 3, in which the *in vivo* synthesis of ^{35}S-labeled AAV proteins at 40°C in cells using either Ad5wt or Ad5ts125 was analyzed. As shown in Fig. 3 (tracks c and d) the AAV VP1, VP2, and VP3 proteins were immunoprecipitated from wild-type helped cells in proportions similar to those observed in purified particles (Fig. 3, track M).

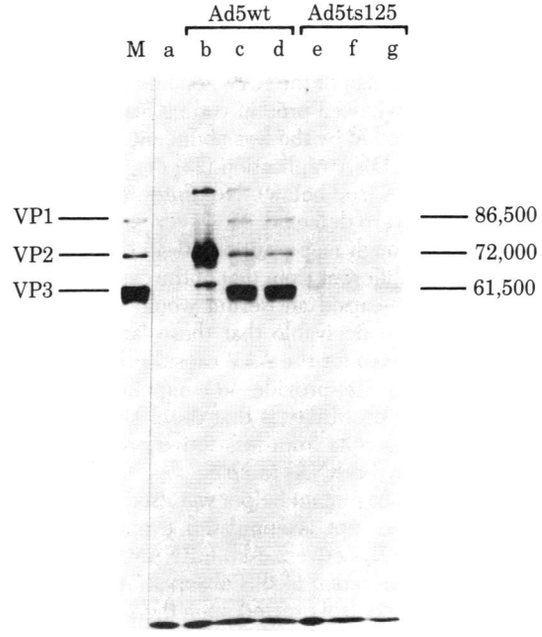

FIG. 3. Analysis of AAV proteins synthesized *in vivo* at 40°C with a temperature-sensitive or wild-type helper. ^{35}S-Labeled AAV proteins from infected cells were detected by immunoprecipitation with AAV antiserum and electrophoresis in 9% acrylamide gels. Track M, ^{35}S-labeled AAV proteins from purified particles. Track a, uninfected cells. Track b, Ad5wt [10 plaque-forming units (PFU)/cell]; track c, Ad5wt + AAV2 (25 IU/cell); track d, Ad5wt + AAV2 (100 IU/cell). Track e, Ad5ts125 (10 PFU/cell); track f, Ad5ts125 + AAV2 (25 IU/cell); track g, Ad5ts125 + AAV2 (100 IU/cell). The molecular weights shown for the AAV proteins are those determined relative to [^{3}H]methyl-labeled proteins obtained from New England Nuclear.

With the mutant helper (Fig. 3, tracks f and g) VP1 and VP2 were undetectable and only a minor band in the position of VP3 was observed. This residual material may not be VP3, because there is a host cell protein from uninfected cells migrating in this position (track a). This cell protein is shutoff in wild-type-infected cells (track b) but not in ts125-infected cells (track e). If this band is in fact VP3, we estimate that VP3 accumulation with the mutant helper (tracks f and g) is less than 1/50th of that with a wild-type helper (tracks c and d). This decrease is of the magnitude previously observed for AAV infectivity or fluorescent antigen when Ad5ts125 was used as the helper at 40°C (9). At the permissive (32°C) or semipermissive (37°C) temperatures there were equal amounts of virus proteins synthesized with either mutant or wild-type helper (data not shown).

In the samples from Ad5wt-infected cells (Fig. 3, track b) there was some nonspecific precipitation of adenovirus proteins (22), including the M_r 72,000 DNA-binding protein (migrating very close to VP2) as well as lesser amounts of hexon protein (migrating slower than VP1) and Ad fiber protein (migrating slightly slower than VP3). Accumulation of these Ad proteins is greatly inhibited by an increasing multiplicity of AAV infection (Fig. 3, tracks c and d; unpublished data) or by the mutation in Ad5ts125. These Ad proteins were also precipitated nonspecifically with normal rabbit serum, whereas AAV proteins were not precipitated by normal serum. Furthermore, no AAV proteins were precipitated by AAV antiserum from cells uninfected or infected with Ad5wt or Ad5ts125. The Ad M_r 72,000 protein precipitation is probably due to formation of aggregates under certain salt conditions (23).

We have also shown that when the Ad5ts125 mutant was the helper at 40°C there was apparently a selective defect in the splicing of certain AAV RNAs, because accumulation of the 3.3-kb and 3.9-kb spliced RNAs was inhibited. However, a normal amount of the 2.3-kb AAV mRNA was accumulated (9). Because the 2.3-kb AAV RNA specifies the VP1, VP2, and VP3 proteins, the failure to accumulate these proteins with the mutant helper (9) does not appear to be directly accounted for by the effect of the same mutant helper on the larger spliced AAV RNAs. There are at least two possible explanations for these observations. Adenovirus early region 2 may exert, directly or indirectly, translational control on the AAV 2.3-kb RNA. Alternatively, the 2.3-kb RNA synthesized with the mutant helper may have an altered structure that prevents efficient translation.

Several lines of evidence argue against the second of these two hypotheses. First, no obvious differences in the splicing or polyadenylylation of the 2.3-kb RNA was detected. However, the procedures used would not detect small changes in the splicing that could affect the initiator codon or the reading frame of the mRNA. Second, analysis of methyl caps in AAV RNA isolated from cells using either an Ad5wt or an Ad5ts125 helper at 40°C showed identical structures (unpublished).

We also compared the biological activity of the 2.3-kb AAV RNA from wild-type- or mutant-helped cells grown at 40°C. Equal numbers of cells infected with the ts125 mutant or wild-type Ad were grown and RNAs were purified in parallel. When equal cell equivalents of the RNA were analyzed by the Berk and Sharp procedure (15) there appeared to be equivalent amounts of the 2.3-kb RNA in the two preparations, but the mutant-helped RNA was clearly deficient in the 3.3-kb and 3.9-kb species (Fig. 4A). Equal cell equivalents of the two RNA preparations were also sedimented in sucrose gradients and equal aliquots of alternate fractions were incubated in the *in vitro* translation system. As shown in Fig. 4 B and C, both RNA preparations directed *in vitro* synthesis of AAV capsid proteins with similar efficiencies. Thus the biological activity of the 2.3-kb RNA in an *in vitro* translation system does not seem to be

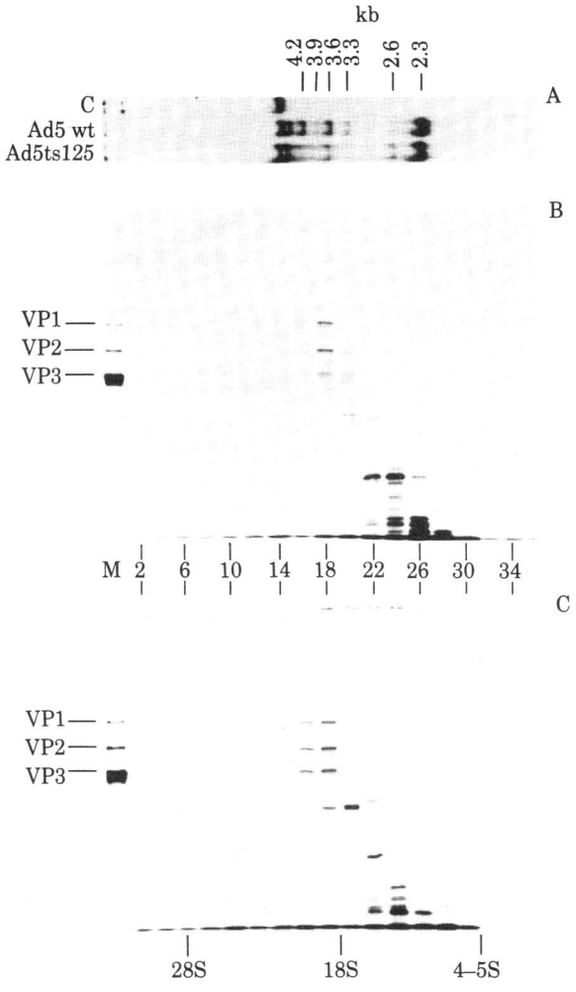

FIG. 4. *In vitro* translation of AAV mRNA from wild-type- or mutant-helped cells. Cytoplasmic poly(A)-containing RNA was prepared from two cultures of KB cells infected with AAV2 (25 IU/cell) and either Ad5wt (10 PFU/cell) or Ad5ts125 (10 PFU/cell) as helper and grown at 40°C. (*A*) Berk and Sharp analysis of the two RNA preparations. Equal aliquots of the RNA preparations were annealed with denatured AAV [^{32}P]DNA and analyzed as for Fig. 2*A*. Individual tracks contained RNA from mutant- or wild-type-helped cells or a control yeast RNA (*C*). The sizes of the RNAs were determined as in Fig. 2. (*B* and *C*) Equal cell equivalents (100 μg) of the mutant- (*B*) or wild-type- (*C*) helped RNAs were sedimented through neutral sucrose gradients and RNA from even-numbered fractions was translated *in vitro* and analyzed as described in the legend to Fig. 2*B*. Tracks 12–28 indicate the sucrose gradient fraction number. Track M shows ^{35}S-labeled AAV capsid proteins from purified AAV particles. In parallel sucrose gradients, 28S, 18S, and 4–5S RNA ran in in fractions 6, 19, and 32 respectively.

impaired by the Ad5ts125 mutation. The lower molecular weight products synthesized from more slowly sedimenting fractions probably represent translation of partially degraded RNA molecules.

These experiments support the concept that Ad may exert a translational control over the production of AAV capsid proteins. This control appears to be influenced by mutations in the Ad early region 2 gene for the M_r 72,000 protein.

DISCUSSION

The work reported here shows that the 2.3-kb AAV mRNA in an *in vitro* translation system specifies all three AAV capsid proteins. This message has a leader of approximately 50 nucleotides located at map position 39.5 to 40.5 and a main body between map position 48 and 96 (6). Thus the AAV capsid proteins are specified by the right-hand half of the genome only. Because the main body of AAV RNA is approximately 2.3 kb, nearly all of this RNA would be required to code for an M_r 85,000 VP1 protein. It was suggested from *in vivo* radioactive labeling of AAV proteins that VP2 and VP3 were derived by proteolytic cleavage of VP1 at the level of the nascent polypeptide (24). While our results are consistent with this idea, we did not detect the M_r 25,000 and M_r 16,000 nonstructural polypeptides that were suggested to be the cleavage products of the capsid protein processing (24). These smaller proteins are not AAV specific, are not precipitated by our antiserum, or are derived from broken AAV mRNA. Alternatively, they may be unstable in the *in vitro* translation system. Additionally, in the absence of complete AAV mRNA sequence data, we cannot rigorously eliminate the possibility that the VP1, VP2, and VP3 proteins are derived from 2.3-kb RNAs that have minor variations in their splicing patterns or their 5′ start points. In agreement with Buller and Rose (24), our results provide no evidence for a M_r 120,000 (VP0) protein that was suggested as a precursor to VP1, VP2, and VP3 (3).

The VP1, VP2, and VP3 proteins were accumulated in the *in vitro* system in ratios different from those observed for the *in vivo* labeled proteins or purified AAV particles (see Figs. 3 and 4 *B* and *C*). This suggests that the efficiency of processing in the reticulocyte system is different from that in the normal host cells. It is interesting that translation of AAV RNA by microinjection into *Xenopus* oocytes results in accumulation of VP1, VP2, and VP3 in ratios similar to those of the normal host cells (unpublished). Whether the apparent processing of AAV capsid proteins is catalyzed by a cellular enzyme(s) or is an autocatalytic process is unknown. It is also not known if this processing occurs at the amino or carboxyl terminus.

The presence of additional cytoplasmic AAV RNAs that extend through the left half of the AAV genome (Fig. 1) suggests that this region may have a protein-coding function. It is possible that proteins coded by the less-abundant AAV RNAs may have a role in AAV DNA replication (25) or perhaps in translation of AAV mRNA (see below). No proteins specific for the larger AAV RNAs were detected. However, any such proteins may not extend through map position 40–48 or may do so but in a reading frame different from that of the capsid proteins. In either case our anti-capsid antiserum would not detect such proteins. It is also conceivable that these larger AAV RNAs might also code *in vivo* for the AAV capsid proteins, although our *in vitro* experiments provide no compelling evidence for this. An alternative possibility is that these larger AAV RNAs may represent transcripts from less-active promoters and are not used as functional mRNAs *in vivo*.

When the Ad5ts125 mutant helper was used at 40°C the AAV capsid proteins were not accumulated even though normal amounts of biologically active 2.3-kb mRNA were synthesized. The simplest interpretation of this observation is that there is a translational level control exerted upon the 2.3-kb mRNA. An alternative possibility, which is difficult to rigorously eliminate, is that the Ad mutation results in a normal rate of synthesis but greatly reduced stability of VP1, VP2, and VP3. However, *in vivo* pulse–chase labeling experiments provided no evidence for such a possibility (unpublished).

How the mutation in the Ad M_r 72,000 DNA-binding protein affects translation of AAV mRNA is not clear. This protein has already been shown to have a number of pleiotropic affects, including a down modulation of some Ad early mRNA (26, 27), a stoichiometric involvement in Ad DNA replication (10, 11), and an effect on splicing of certain Ad (28) and AAV (9) mRNAs.

The effect of the mutation upon translation of AAV 2.3-kb mRNA may be indirect via an interaction with some cellular component. One most interesting possibility is that the larger AAV RNAs, which are not made in the presence of the mutant helper, may code for an AAV product required for efficient translation *in vivo* of the AAV 2.3-kb mRNA. It is conceivable that the M_r 72,000 protein is not required for AAV but that the ts125 mutation results in interference with certain other potential AAV helper functions expressed by Ad. One such example might be the failure to turn off Ad early region 4 expression (26, 27). Whatever the mechanism, our experiments describe a temperature-sensitive system that may prove extremely useful in analyzing an apparent translation control mechanism exerted on a eukaryotic mRNA. This appears to be one of the few examples of a temperature-sensitive mutation in a eukaryotic protein that affects translation of an mRNA *in vivo*.

An additional feature of this work is that AAV may provide a probe to dissect several pleiotropic functions of the M_r 72,000 protein, because the Ad DNA replication function of this protein is not required for AAV DNA replication (29). Thus, the properties of M_r 72,000 protein manifested later in the Ad replication cycle (i.e., after Ad DNA replication begins) can be assayed in human cells by using AAV as the probe. For Ad, these functions can be directly assayed only in a host range system.

The growth of human adenoviruses is restricted in most monkey cells (30). This host restriction is exerted in part at the level of splicing of certain Ad late gene mRNAs (28). Also, there may be a defect in translation of Ad late gene mRNA, but evidence for this is more controversial (31–33). The fractional protein of simian virus 40 tumor (T) antigen induced by the nondefective Ad2/simian virus 40 hybrid ($Ad2ND_2$) that allows Ad to overcome this restriction in simian cells has been shown to bind specifically to the 40S ribosomal subunit *in vivo* and *in vitro* (34). AAV grown in African green monkey kidney cells by using a human Ad helper exhibited a similar restriction; AAV RNA was synthesized but no AAV proteins accumulated. This was taken to indicate translation level control on AAV mRNA (35). These results appear to be in accord with our observations, because it is now known that the host-range restriction for human adenoviruses in monkey cells is controlled by the Ad M_r 72,000 protein. Certain human adenoviruses with mutations mapping in this protein efficiently overcome the monkey cell restriction (36, 37). One such mutant, Ad2hr400, helps AAV efficiently in TC7 cells (a subline of African green monkey kidney cells), which are normally restrictive for both Ad and AAV (unpublished).

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